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SEQUENCE LISTING

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<120> A TRUNCATED FORM OF FIBROBACTER SUCCINOGENES 1, 3-1,
4-BETA-D-GLUCANASE WITH IMPROVED ENZYMATIC ACTIVITY AND
THERMO-TOLERANCE

<130> 4910-8

<140> 09/654,652

<141> 2000-09-05

<160> 11

<170> PatentIn Ver. 2.1

<210> 1

<211> 248

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Modified enzyme
with enhanced activity and thermal stability

<400> 1

Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu
1 5 10 15

Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala
20 25 30

Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile
35 40 45

Ala Asp Gly Arg Pro Trp Val Glu Val Asp Ile Glu Val Leu Gly Lys
50 55 60

Asn Pro Gly Ser Phe Gln Ser Asn Ile Ile Thr Gly Lys Ala Gly Ala
65 70 75 80

Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln
85 90 95

Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp
100 105 110

Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gly Gln Val Ser
115 120 125

Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu
130 135 140

Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe
145 150 155 160

Gln Phe Ile Asn Trp Val Lys Val Tyr Lys Tyr Thr Pro Gly Gln Gly
 165 170 175

Glu Gly Gly Ser Asp Phe Thr Leu Asp Trp Thr Asp Asn Phe Asp Thr
 180 185 190

Phe Asp Gly Ser Arg Trp Gly Lys Gly Asp Trp Thr Phe Asp Gly Asn
 195 200 205

Arg Val Asp Leu Thr Asp Lys Asn Ile Tyr Ser Arg Asp Gly Met Leu
 210 215 220

Ile Leu Ala Leu Thr Arg Lys Gly Gln Glu Ser Phe Asn Gly Gln Val
 225 230 235 240

Pro Arg Asp Asp Glu Pro Ala Pro
 245

<210> 2

<211> 267

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Modified enzyme
 with enhanced activity and thermal stability

<400> 2

Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu
 1 5 10 15

Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala
 20 25 30

Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile
 35 40 45

Ala Asp Gly Arg Pro Trp Val Glu Val Asp Ile Glu Val Leu Gly Lys
 50 55 60

Asn Pro Gly Ser Phe Gln Ser Asn Ile Ile Thr Gly Lys Ala Gly Ala
 65 70 75 80

Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln
 85 90 95

Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp
 100 105 110

Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gly Gln Val Ser
 115 120 125

Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu
 130 135 140

Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe
 145 150 155 160
 Gln Phe Ile Asn Trp Val Lys Val Tyr Lys Tyr Thr Pro Gly Gln Gly
 165 170 175
 Glu Gly Gly Ser Asp Phe Thr Leu Asp Trp Thr Asp Asn Phe Asp Thr
 180 185 190
 Phe Asp Gly Ser Arg Trp Gly Lys Gly Asp Trp Thr Phe Asp Gly Asn
 195 200 205
 Arg Val Asp Leu Thr Asp Lys Asn Ile Tyr Ser Arg Asp Gly Met Leu
 210 215 220
 Ile Leu Ala Leu Thr Arg Lys Gly Gln Glu Ser Phe Asn Gly Gln Val
 225 230 235 240
 Pro Arg Asp Asp Glu Pro Ala Pro Asn Ser Ser Ser Val Asp Lys Leu
 245 250 255
 Ala Ala Ala Leu Glu His His His His His
 260 265

<210> 3

<211> 349

<212> PRT

<213> Fibrobacter succinogenes

<400> 3

Met Asn Ile Lys Lys Thr Ala Val Lys Ser Ala Leu Ala Val Ala Ala
 1 5 10 15

Ala Ala Ala Ala Leu Thr Thr Asn Val Ser Ala Lys Asp Phe Ser Gly
 20 25 30

Ala Glu Leu Tyr Thr Leu Glu Glu Val Gln Tyr Gly Lys Phe Glu Ala
 35 40 45

Arg Met Lys Met Ala Ala Ala Ser Gly Thr Val Ser Ser Met Phe Leu
 50 55 60

Tyr Gln Asn Gly Ser Glu Ile Ala Asp Gly Arg Pro Trp Val Glu Val
 65 70 75 80

Asp Ile Glu Val Leu Gly Lys Asn Pro Gly Ser Phe Gln Ser Asn Ile
 85 90 95

Ile Thr Gly Lys Ala Gly Ala Gln Lys Thr Ser Glu Lys His His Ala
 100 105 110

Val Ser Pro Ala Ala Asp Gln Ala Phe His Thr Tyr Gly Leu Glu Trp
 115 120 125

Thr Pro Asn Tyr Val Arg Trp Thr Val Asp Gly Gln Glu Val Arg Lys
 130 135 140

Thr Glu Gly Gly Gln Val Ser Asn Leu Thr Gly Thr Gln Gly Leu Arg
 145 150 155 160
 Phe Asn Leu Trp Ser Ser Glu Ser Ala Ala Trp Val Gly Gln Phe Asp
 165 170 175
 Glu Ser Lys Leu Pro Leu Phe Gln Phe Ile Asn Trp Val Lys Val Tyr
 180 185 190
 Lys Tyr Thr Pro Gly Gln Gly Glu Gly Ser Asp Phe Thr Leu Asp
 195 200 205
 Trp Thr Asp Asn Phe Asp Thr Phe Asp Gly Ser Arg Trp Gly Lys Gly
 210 215 220
 Asp Trp Thr Phe Asp Gly Asn Arg Val Asp Leu Thr Asp Lys Asn Ile
 225 230 235 240
 Tyr Ser Arg Asp Gly Met Leu Ile Leu Ala Leu Thr Arg Lys Gly Gln
 245 250 255
 Glu Ser Phe Asn Gly Gln Val Pro Arg Asp Asp Glu Pro Ala Pro Gln
 260 265 270
 Ser Ser Ser Ala Pro Ala Ser Ser Ser Val Pro Ala Ser Ser
 275 280 285
 Ser Ser Val Pro Ala Ser Ser Ser Ala Phe Val Pro Pro Ser Ser
 290 295 300
 Ser Ser Ala Thr Asn Ala Ile His Gly Met Arg Thr Thr Pro Ala Val
 305 310 315 320
 Ala Lys Glu His Arg Asn Leu Val Asn Ala Lys Gly Ala Lys Val Asn
 325 330 335
 Pro Asn Gly His Lys Arg Tyr Arg Val Asn Phe Glu His
 340 345

<210> 4
 <211> 744
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: DNA encoding a
 modified enzyme

<220>
 <221> CDS
 <222> (1)..(744)

<400> 4
 atg gtt agc gca aag gat ttt agc ggt gcc gaa ctc tac acg tta gaa
 Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu
 1 5 10 15

gaa gtt cag tac ggt aag ttt gaa gcc cgt atg aag atg gca gcc gca Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala	20	25	30	96
tcg gga aca gtc agt tcc atg ttc ctc tac cag aat ggt tcc gaa atc Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile	35	40	45	144
gcc gat gga agg ccc tgg gta gaa gtg gat att gaa gtt ctc ggc aag Ala Asp Gly Arg Pro Trp Val Glu Val Asp Ile Glu Val Leu Gly Lys	50	55	60	192
aat ccg ggc agt ttc cag tcc aac atc att acc ggt aag gcc ggc gca Asn Pro Gly Ser Phe Gln Ser Asn Ile Ile Thr Gly Lys Ala Gly Ala	65	70	75	240
caa aag act agc gaa aag cac cat gct gtt agc ccc gcc gcc gat cag Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln	85	90	95	288
gct ttc cac acc tac ggt ctc gaa tgg act ccg aat tac gtc cgc tgg Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp	100	105	110	336
act gtt gac ggt cag gaa gtc cgc aag acg gaa ggt ggc cag gtt tcc Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gly Gln Val Ser	115	120	125	384
aac ttg aca ggt aca cag gga ctc cgt ttt aac ctt tgg tcg tct gag Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu	130	135	140	432
agt gcg gct tgg gtt ggc cag ttc gat gaa tca aag ctt ccg ctt ttc Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe	145	150	155	480
cag ttc atc aac tgg gtc aag gtt tat aag tat acg ccg ggc cag ggc Gln Phe Ile Asn Trp Val Lys Val Tyr Lys Tyr Thr Pro Gly Gln Gly	165	170	175	528
gaa ggc ggc agc gac ttt acg ctt gac tgg acc gac aat ttt gac acg Glu Gly Ser Asp Phe Thr Leu Asp Trp Thr Asp Asn Phe Asp Thr	180	185	190	576
ttt gat ggc tcc cgc tgg ggc aag ggt gac tgg aca ttt gac ggt aac Phe Asp Gly Ser Arg Trp Gly Lys Gly Asp Trp Thr Phe Asp Gly Asn	195	200	205	624
cgt gtc gac ctc acc gac aag aac atc tac tcc aga gat ggc atg ttg Arg Val Asp Leu Thr Asp Lys Asn Ile Tyr Ser Arg Asp Gly Met Leu	210	215	220	672
atc ctc gcc ctc acc cgc aaa ggt cag gaa agc ttc aac ggc cag gtt Ile Leu Ala Leu Thr Arg Lys Gly Gln Glu Ser Phe Asn Gly Gln Val	225	230	235	720
			240	

ccg aga gat gac gaa cct gct ccg	744
Pro Arg Asp Asp Glu Pro Ala Pro	
245	
<210> 5	
<211> 804	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Description of Artificial Sequence: DNA encoding a	
modified enzyme	
<220>	
<221> CDS	
<222> (1)..(804)	
<400> 5	
atg gtt agc gca aag gat ttt agc ggt gcc gaa ctc tac acg tta gaa	48
Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu	
1 5 10 15	
gaa gtt cag tac ggt aag ttt gaa gcc cgt atg aag atg gca gcc gca	96
Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala	
20 25 30	
tcg gga aca gtc agt tcc atg ttc ctc tac cag aat ggt tcc gaa atc	144
Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile	
35 40 45	
gcc gat gga agg ccc tgg gta gaa gtg gat att gaa gtt ctc ggc aag	192
Ala Asp Gly Arg Pro Trp Val Glu Val Asp Ile Glu Val Leu Gly Lys	
50 55 60	
aat ccg ggc agt ttc cag tcc aac atc att acc ggt aag gcc ggc gca	240
Asn Pro Gly Ser Phe Gln Ser Asn Ile Ile Thr Gly Lys Ala Gly Ala	
65 70 75 80	
caa aag act agc gaa aag cac cat gct gtt agc ccc gcc gat cag	288
Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln	
85 90 95	
gct ttc cac acc tac ggt ctc gaa tgg act ccg aat tac gtc cgc tgg	336
Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp	
100 105 110	
act gtt gac ggt cag gaa gtc cgc aag acg gaa ggt ggc cag gtt tcc	384
Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gly Gln Val Ser	
115 120 125	
aac ttg aca ggt aca cag gga ctc cgt ttt aac ctt tgg tcg tct gag	432
Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu	
130 135 140	
agt gcg gct tgg gtt ggc cag ttc gat gaa tca aag ctt ccg ctt ttc	480
Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe	
145 150 155 160	

cag ttc atc aac tgg gtc aag gtt tat aag tat acg ccg ggc cag ggc	528
Gln Phe Ile Asn Trp Val Lys Val Tyr Lys Tyr Thr Pro Gly Gln Gly	
165 170 175	
gaa ggc ggc agc gac ttt acg ctt gac tgg acc gac aat ttt gac acg	576
Glu Gly Gly Ser Asp Phe Thr Leu Asp Trp Thr Asp Asn Phe Asp Thr	
180 185 190	
ttt gat ggc tcc cgc tgg ggc aag ggt gac tgg aca ttt gac ggt aac	624
Phe Asp Gly Ser Arg Trp Gly Lys Gly Asp Trp Thr Phe Asp Gly Asn	
195 200 205	
cgt gtc gac ctc acc gac aag aac atc tac tcc aga gat ggc atg ttg	672
Arg Val Asp Leu Thr Asp Lys Asn Ile Tyr Ser Arg Asp Gly Met Leu	
210 215 220	
atc ctc gcc ctc acc cgc aaa ggt cag gaa agc ttc aac ggc cag gtt	720
Ile Leu Ala Leu Thr Arg Lys Gly Gln Glu Ser Phe Asn Gly Gln Val	
225 230 235 240	
ccg aga gat gac gaa cct gct ccg aat tcg agc tcc gtc gac aag ctt	768
Pro Arg Asp Asp Glu Pro Ala Pro Asn Ser Ser Val Asp Lys Leu	
245 250 255	
gcg gcc gca ctc gag cac cac cac cac cac tga	804
Ala Ala Ala Leu Glu His His His His His His	
260 265	
<210> 6	
<211> 1050	
<212> DNA	
<213> Fibrobacter succinogenes	
<220>	
<221> CDS	
<222> (1)..(1047)	
<400> 6	
atg aac atc aag aaa act gca gtc aag agc gct ctc gcc gta gca gcc	48
Met Asn Ile Lys Lys Thr Ala Val Lys Ser Ala Leu Ala Val Ala Ala	
1 5 10 15	
gca gca gca gcc ctc acc acc aat gtt agc gca aag gat ttt agc ggt	96
Ala Ala Ala Leu Thr Thr Asn Val Ser Ala Lys Asp Phe Ser Gly	
20 25 30	
gcc gaa ctc tac acg tta gaa gaa gtt cag tac ggt aag ttt gaa gcc	144
Ala Glu Leu Tyr Thr Leu Glu Glu Val Gln Tyr Gly Lys Phe Glu Ala	
35 40 45	
cgt atg aag atg gca gcc gca tcg gga aca gtc agt tcc atg ttc ctc	192
Arg Met Lys Met Ala Ala Ala Ser Gly Thr Val Ser Ser Met Phe Leu	
50 55 60	

tac cag aat ggt tcc gaa atc gcc gat gga agg ccc tgg gta gaa gtg	240
Tyr Gln Asn Gly Ser Glu Ile Ala Asp Gly Arg Pro Trp Val Glu Val	
65 70 75 80	
gat att gaa gtt ctc ggc aag aat ccg ggc agt ttc cag tcc aac atc	288
Asp Ile Glu Val Leu Gly Lys Asn Pro Gly Ser Phe Gln Ser Asn Ile	
85 90 95	
att acc ggt aag gcc ggc gca caa aag act agc gaa aag cac cat gct	336
Ile Thr Gly Lys Ala Gly Ala Gln Lys Thr Ser Glu Lys His His Ala	
100 105 110	
gtt agc ccc gcc gcc gat cag gct ttc cac acc tac ggt ctc gaa tgg	384
Val Ser Pro Ala Ala Asp Gln Ala Phe His Thr Tyr Gly Leu Glu Trp	
115 120 125	
act ccg aat tac gtc cgc tgg act gtt gac ggt cag gaa gtc cgc aag	432
Thr Pro Asn Tyr Val Arg Trp Thr Val Asp Gly Gln Glu Val Arg Lys	
130 135 140	
acg gaa ggt ggc cag gtt tcc aac ttg aca ggt aca cag gga ctc cgt	480
Thr Glu Gly Gly Gln Val Ser Asn Leu Thr Gly Thr Gln Gly Leu Arg	
145 150 155 160	
ttt aac ctt tgg tcg tct gag agt gcg gct tgg gtt ggc cag ttc gat	528
Phe Asn Leu Trp Ser Ser Glu Ser Ala Ala Trp Val Gly Gln Phe Asp	
165 170 175	
gaa tca aag ctt ccg ctt ttc cag ttc atc aac tgg gtc aag gtt tat	576
Glu Ser Lys Leu Pro Leu Phe Gln Phe Ile Asn Trp Val Lys Val Tyr	
180 185 190	
aag tat acg ccg ggc cag ggc gaa ggc ggc agc gac ttt acg ctt gac	624
Lys Tyr Thr Pro Gly Gln Gly Glu Gly Ser Asp Phe Thr Leu Asp	
195 200 205	
tgg acc gac aat ttt gac acg ttt gat ggc tcc cgc tgg ggc aag ggt	672
Trp Thr Asp Asn Phe Asp Thr Phe Asp Gly Ser Arg Trp Gly Lys Gly	
210 215 220	
gac tgg aca ttt gac ggt aac cgt gtc gac ctc acc gac aag aac atc	720
Asp Trp Thr Phe Asp Gly Asn Arg Val Asp Leu Thr Asp Lys Asn Ile	
225 230 235 240	
tac tcc aga gat ggc atg ttg atc ctc gcc ctc acc cgc aaa ggt cag	768
Tyr Ser Arg Asp Gly Met Leu Ile Leu Ala Leu Thr Arg Lys Gly Gln	
245 250 255	
gaa agc ttc aac ggc cag gtt ccg aga gat gac gaa cct gct ccg caa	816
Glu Ser Phe Asn Gly Gln Val Pro Arg Asp Asp Glu Pro Ala Pro Gln	
260 265 270	
tct tct agc agc gct ccg gca tct tct agc agt gtt ccg gca agc tcc	864
Ser Ser Ser Ala Pro Ala Ser Ser Ser Ser Val Pro Ala Ser Ser	
275 280 285	

tct	agc	gtc	cct	gcc	tcc	tcg	agc	agc	gca	ttt	gtt	ccg	ccg	agc	tcc	912
Ser	Ser	Val	Pro	Ala	Ser	Ser	Ser	Ser	Ala	Phe	Val	Pro	Pro	Ser	Ser	
290					295							300				
tcg	agc	gcc	aca	aac	gca	atc	cac	gga	atg	cgc	aca	act	ccg	gca	gtt	960
Ser	Ser	Ala	Thr	Asn	Ala	Ile	His	Gly	Met	Arg	Thr	Thr	Pro	Ala	Val	
305					310				315						320	
gca	aag	gaa	cac	cgc	aat	ctc	gtg	aac	gcc	aag	ggt	gcc	aag	gtg	aac	1008
Ala	Lys	Glu	His	Arg	Asn	Leu	Val	Asn	Ala	Lys	Gly	Ala	Lys	Val	Asn	
325								330							335	
ccg	aat	ggc	cac	aag	cgt	tat	cgc	gtg	aac	ttt	gaa	cac	taa			1050
Pro	Asn	Gly	His	Lys	Arg	Tyr	Arg	Val	Asn	Phe	Glu	His				
340								345								
<210> 7																
<211> 23																
<212> DNA																
<213> Artificial Sequence																
<220>																
<223> Description of Artificial Sequence: Primer																
<400> 7																
tcaccaccat ggttagcgca aag																23
<210> 8																
<211> 25																
<212> DNA																
<213> Artificial Sequence																
<220>																
<223> Description of Artificial Sequence: Primer																
<400> 8																
ggcacgaatt ctgttcaaag ttcac																25
<210> 9																
<211> 27																
<212> DNA																
<213> Artificial Sequence																
<220>																
<223> Description of Artificial Sequence: Primer																
<400> 9																
cagccggcga tggccatgggt tagcgca																27
<210> 10																
<211> 29																
<212> DNA																
<213> Artificial Sequence																

<220>
<223> Description of Artificial Sequence: Primer

<400> 10
ctgctagaag aattcggagc aggttcgtc 29

<210> 11
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
illustrative peptide

<220>
<221> MOD_RES
<222> (2)
<223> An uncharged residue, such as Alanine, Proline or
Glutamine

<400> 11
Pro Xaa Ser Ser Ser Ser
1 5